CHAPTER - III
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MATERIALS AND METHODS

This chapter deals with the description of the materials used and methods or techniques adopted during the course of study.

3.1 Study area – Raipur city, capital of Chhattisgarh state.

3.2 Geographical situation, climate and weather conditions

Raipur is situated in the mid-eastern part of the Chhattisgarh state and lies at 21° 16' N Latitude and 81° 36 E' longitude with an altitude of 298.5 metres above the mean sea level. The region comes under sub-humid conditions. The average annual rainfall of the area is 1150 mm and major amount of precipitation occurs between June - September (3-4 months), hottest and coolest months are May and December respectively.

3.3 Site–Outskirts of Raipur city towards Bilaspur at Raipur-Bilaspur highway.

In Raipur, a wide stretch of Lands along the railway track and highway are used for cultivation of crops specially vegetables. The area is receiving untreated sewage irrigation for the last 25-30 years. Farmers are growing leafy vegetables like spinach, red leafy vegetable (bhaji), karmata (bhaji) besides tomatoes, brinjals, okra, onion, etc. Some farmers with comparatively large area are cultivating paddy also. Vegetables are being grown round the year since there is no scarcity of water for irrigation as untreated city sewage water is available for the purpose. Among leafy vegetables, mainly bhajis are consumed by rural poor of the adjoining area and produce have market in nearby peri-urban area of the city. In a survey of these soils, eight farmers’
fields were selected as study site. The soil of experimental site was characterized as sandy clay loam (Sand 48.3%, silt 23.4% and clay 28.3%). The bulk density of the soil was calculated to be 1.24 g/cm$^3$, particle density 2.34 g/cm$^3$ and porosity 47 per cent.

3.4 Collection and storage of samples

3.4.1 Sewage effluent water

Sewage water/effluent samples were collected from three points and marked as site I, II and III respectively. Samples were collected and stored in clean 2 L plastic/poly propylene bottles for investigations.

3.4.2 Soil

Sixteen surface (0-15 cm) soil samples were collected from sewage effluent irrigated fields. Besides, same number of surface (0-15 cm) soil samples were also collected from adjacent fields, not receiving sewage effluent irrigation. Soil samples were air dried ground with hand using pestle and mortar and passed through 2 mm polythene sieve and stored in polythene bags for investigations.

3.4.3 Soil profile

Four sites were selected receiving sewage irrigation and one not receiving sewage irrigation. Soil profile samples were taken using soil tube auger at 0-15, 15-30 and 30-50 cm depths. These soil samples were air dried ground with hand using pestle and mortar and passed through 2 mm polythene sieve and stored in polythene bags for investigations.
3.4.4 Crops

Representative samples of matured leaves and fruits of vegetables namely, spinach, red leafy vegetable (bhaji), tomatoes, okra, brinjal along with paddy straw and grains were collected which were grown on sewage effluent irrigated soils. All these vegetables, paddy straw and grains were also collected from adjacent sites which do not receive sewage effluent water irrigation. Plant samples were washed with tap water and then with distilled water, dried in an electric oven at 60°C for 72 hours and ground to make powder using stainless steel Wiley mill and stored in air tight lockable polythene bags for investigations.

3.5 Analysis of sewage effluent water

In laboratory water samples were analyzed for pH, EC, TS, TDS, TSS, concentration of cations and anions namely, Na⁺, K⁺, Ca²⁺, Mg²⁺, N, P, Cl⁻, SO₄²⁻, CO₃⁻, HCO₃⁻, micronutrients and metals viz, Zn, Fe, Cu, Mn, Ni, Cr, Pb and Cd. The values of Sodium Adsorption Ratio (SAR) was calculated using values of certain cations following standard formula procedure. 

*Sewage effluent water after filtering through Whatman No. 1 filter paper was used in all the determinations.*

3.5.1 Determination of pH (Chapman and Pratt, 1961)

The pH of the water sample was determined by digital pH meter taking 50 ml water sample in 100 ml clean beaker after calibrating the pH meter using standard buffer solutions of pH 4.0 and 9.2.
3.5.2 Determination of electrical conductivity (Tandon, 1993)

The electrical conductivity was measured using digital Conductivity meter. Fifty ml water sample was taken in a 100 ml clean beaker. The electrodes of the conductivity meter were washed with distilled water, wiped dried with filter paper and immersed in the water sample. The EC was recorded directly in terms of dS m⁻¹.

3.5.3 Determination of Chloride (AOAC, 1950)

Mohr's titration method is most commonly used for chloride estimation. It depends upon the formation of a sparingly-soluble brick red precipitate of silver chromate (AgCrO₄) at the end point when sample is titrated against standard silver nitrate solution in presence of potassium chromate (K₂CrO₄) as indicator.

Reagents

- Potassium chromate indicator solution 5% by dissolving 5 g of K₂CrO₄ in distilled water and volume made up to 100 ml.
- Standard silver nitrate solution 0.05 N by dissolving 8.494 g of AR grade AgNO₃ in distilled water and volume made up to 1 litre and stored in amber colored bottle.
- Standard sodium chloride solution 0.05 N by dissolving 2.925 g NaCl in distilled water and volume made up to 1 litre.

Procedure

Taken 25 ml water sample in an 150 ml Erlenmeyer flask and diluted with 25 ml distilled water. After adding 5-6 drops of potassium chromate indicator, the contents were titrated with standard silver nitrate solution till a
brick red colored precipitate appears. A blank was also run using distilled water. Silver nitrate solution was standardized using standard sodium chloride solution. After calculations the results were expressed as Cl⁻ mg L⁻¹.

3.5.4 Determination and carbonates and bicarbonates (AOAC, 1950)

Sum of the carbonate (CO₃²⁻) and bicarbonate (HCO₃⁻) ions constitute alkalinity of the water as temporary hardness and raises its pH to more than 7.5. Carbonate and bicarbonate in the sample were determined by titrating it against standard sulphuric acid solution using phenolphthalein and methyl orange as indicators. Addition of phenolphthalein gives red color in presence of carbonates and titration with H₂SO₄ converts CO₃²⁻ into HCO₃⁻ and decolorizes the red color. In this colorless solution, methyl orange is added which gives yellow color. Further titration against H₂SO₄ neutralizes all the HCO₃⁻ (originally present and those converted from CO₃²⁻) into H₂O and CO₂ and the color changes from yellow to rosy red.

Reagents

- Standard sulphuric acid solution 0.05 N by diluting 1.4 ml concentrated H₂SO₄ (36 N) to 1 litre with distilled water.
- Standard sodium carbonate solution 0.1 N by dissolving 0.530 g AR grade Na₂CO₃ in distilled water and volume made up to 1 litre.
- Methyl orange indicator solution 0.5% by dissolving 0.5 g methyl orange powder in 100 ml of 95% ethanol.
- Phenolphthalein indicator solution 0.25% by dissolving 0.25 g phenolphthalein powder in 100 ml of 60% ethanol.
**Procedure**

Taken 25 ml water sample in an 150 ml Erlenmeyer flask and diluted with 25 ml distilled water. Added 2-3 drops of phenolphthalein indicator. Red color appears. The contents were titrated against standard sulphuric acid solution till red color disappears. The volume of H$_2$SO$_4$ used at this point was recorded. Now added 2-3 drops of methyl orange into the same flask and again titrated with standard sulphuric acid solution till the color changes into rosy red. Volume of H$_2$SO$_4$ used was again recorded. Contents of carbonate and bicarbonate calculated and expressed in terms of mg L$^{-1}$.

**3.5.5 Determination of Sulphates (Chesnin and Yien, 1950)**

Sulphate contents are determined by the extent of turbidity created by precipitated colloidal barium sulphate suspension. Fine suspension of barium chloride is established by gram acacia, its absorbance is measured after 30 minutes at 420 nm wave length on a spectrophotometer.

**Reagents**

- Sodium acetate-acetic acid buffer by dissolving 25 g sodium acetate in distilled water. Add 7.8 ml of glacial acetic acid and volume made upto 250 ml with distilled water (pH - 4.8).
- Gum acacia (0.25%) by dissolving 0.25 g gum acacia in distilled water and diluted to 100 ml. The solution is filtered through Whatman No.1 filter paper after 24 hours for use.
- Barium chloride crystals ground to pass through 0.5 mm sieve.
- Potassium sulphate – 50 ppm sulphate-S solution by dissolving 0.2722 g of K₂SO₄ AR grade in distilled water and volume made up to 100 ml.

**Procedure**

Five ml water sample is taken in a 25 ml volumetric flask, added 10 ml of sodium acetate-acetic acid buffer to maintain the pH around 4.8. Now added 1 ml of gum acacia and 1 g barium chloride crystals. The flask was swirled till the crystals dissolved then the contents were diluted to 25 ml mark with distilled water. Pipette out 0, 2, 4, 6 and 8 ml of 50 ppm SO₄-S solution into separate 25 ml volumetric flask. To each flask 10 ml of sodium acetate-acetic buffer solution is added and then 1 ml of 0.25% gum acacia solution. Contents were mixed and 1 g barium chloride crystals were added. After swirling the contents solution was diluted to 25 ml mark with distilled water. Measured the turbidity developed with standard SO₄-S solution and prepared the standard calibration curve by measuring the absorbance on spectrophotometer. By the factor of straight line curve in units of absorbance against concentration of SO₄-S, the contents of SO₄-S in samples of water was obtained in mg L⁻¹.

**3.5.6 Determination of calcium and magnesium (Chang and Bray, 1951; Jackson, 1973).**

Ethylene diamine tetraacetic acid (EDTA) forms complexes with Ca and Mg. Titration in presence of Ca-indicator (Ammonium purpurate) determines Ca and in presence of Mg-indicator (Eriochrome Black T) determines Ca + Mg. As Ca-EDTA complex has higher stability than Mg-
EDTA complex, the Mg is determined by subtraction of values of Ca from that of Ca + Mg.

Reagents

• Standard Ca-solution 0.01 N by dissolving 0.5 g AR grade dried CaCO₃ in 10 ml of 0.2 N HCl. Heat the solution to boil, cooled and made up the volume to 1 litre with distilled water. This solution was used for standardizing the EDTA solution.

• EDTA solution 0.01 N by dissolving 2.0 g EDTA sodium salt in distilled water and volume made up to 1 litre.

• Ammonium purpurate (Murexide powder) by weighing 0.2 g of ammonium purpurate and 40 g of potassium sulphate. Both the reagents are mixed thoroughly with pestle and mortar and stored in powdered form.

• Eriochrome Black T indicator solution by weighing 0.5 g of EBT and 4.5 g of hydroxylamine hydrochloride and dissolved both in 100 ml of ethyl alcohol.

• Sodium hydroxide solution 4 N by dissolving 160 g NaOH in distilled water and volume made up to 1 litre.

• Ammonium chloride-ammonium hydroxide buffer solution by dissolving 67.5 g ammonium chloride in 750 ml of concentrated ammonia and volume made up to 1 litre with distilled water.

Procedure

a) Calcium – In a Erlenmeyer flask 5 ml of water sample was taken and diluted by adding 25 ml of distilled water. Five ml of NaOH and 25 mg
of Murexide indicator powder was added. Contents were swirled, and orange red color appeared. The contents were titrated against standard EDTA solution till color changes to lavender purple. Noted the volume of EDTA used.

b) Calcium plus Magnesium – Five ml water sample taken in another clean Erlenmeyer flask and diluted by adding 25 ml distilled water. One ml of NH₄Cl-NH₄OH buffer solution and 3-4 drops of EBT indicator was added. This gave a wine red color. The contents were titrated against standard EDTA solution till color changes to blue or bluish green at the end point. The volume of EDTA used was also noted. The contents of Ca and Ca + Mg were calculated in terms of mg L⁻¹. The value of Mg was obtained by subtracting the value of Ca from the value of Ca + Mg.

3.5.7 Determination of Sodium (Richards, 1954).

Sodium is determined by Flame photometer. When a solution of a salt is sprayed into the flame, the energy of the flame excites the atoms to higher energy levels. When electrons returns back to ground state, they emit radiation of characteristics wave length. The intensity of these radiations is proportional to the concentration of particular element in solution which is measured through photocell of the Flame photometer.

Reagents

- Standard Sodium Solution 1000 ppm Na⁺ by dissolving 2.521 g AR grade NaCl in distilled water and volume made up to 1 litre.
• Standard Working Solution of Na⁺ by diluting 0, 2, 4, 6, 8 and 10 ml of 1000 ppm Na⁺ solution to 500 ml using distilled water. This solution provides 0, 4, 8, 12, 16 and 20 ppm of Na solution.

Procedure

The flame photometer was calibrated by spraying with 0 and 20 ppm Na⁺ solution in reading range of 0-100 on scale. Now serials of dilution of 4, 8, 12 and 16 ppm Na solution are sprayed into the flame. The galvanometer readings were recorded for each spraying. A curve was drawn by plotting flame photometer readings against concentrations of Na⁺. The concentration of Na⁺ in water sample was then read from the curve and reported in terms of mg L⁻¹ of Na.

3.5.8 Determination of Potassium

Potassium was determined by Flame photometer as per the procedure detailed for Na, except that the Na filter was replaced by K filter in the Flame photometer.

Reagents

• Standard Potassium Solution 1000 ppm K by dissolving 1.900 g AR grade KCl in distilled water and volume made up to 1 litre.

• Standard Working Solution of K by diluting 0, 2, 4, 6, 8 and 10 ml of 1000 ppm K solution to 500 ml using distilled water. This solution provides 0, 4, 8, 12, 16 and 20 ppm of K solution.

Procedure

The Na filter in the flame photometer was replaced by K filter and the instrument was calibrated by spraying with 0 and 20 ppm K solution in reading
range of 0-100 on scale. Now serials of dilution of 4, 8, 12 and 16 ppm K solution were sprayed into the flame. The galvanometer readings were recorded for each spraying. A curve was drawn by plotting Flame photometer readings against concentrations of K. The concentration of K in the water sample was then read from the curve and reported in terms of mg L$^{-1}$ of K.

3.5.9 Determination of Phosphorus (Watanabe and Olsen, 1965).

Ammonium molybdate and potassium antimony tartarate react in acid medium with orthophosphates to form phosphomolybdic acid which is reduced to molybdenum blue by ascorbic acid. The intensity of blue color is read on a Photoelectric colorimeter.

Reagents

- Solution A – One g of ammonium molybdate and 0.02 g of potassium antimony tartarate were taken in 1000 ml volumetric flask. Slowly added 16 ml of concentrated H$_2$SO$_4$. Then the contents were dissolved by adding distilled water and volume made up to the mark.

- Solution B – Weighed 0.88 g of ascorbic acid and dissolved in 1 litre of solution A.

- Standard phosphate solution 50 ppm P by dissolving 0.2195 g of anhydrous KH$_2$PO$_4$ in distilled water and volume made to 1 litre.

Procedure

Prepared working solution by series of dilution of standard P solution containing 0, 2, 4, 6, 8 and 10 ppm from 50 ppm P solution into 50 ml volumetric flask and volume made to the mark with distilled water. Ten ml each from the working solution were taken in 50 ml conical flask added 10 ml
of solution B and volume made up to 50 ml with distilled water. A blue color develops in 30 minutes. A blank was also prepared without P contents. The photoelectric colorimeter was adjusted to 100% transmittance with distilled water before start of reading samples. The absorbance of blue color was measured at 660 nm wavelength. A curve was drawn by plotting absorbance against concentration of P in the solution. The relationship is a straight line in plotted curve. From the curve, the concentration of unknown water sample was calculated in terms of mg L⁻¹.

3.5.10 Determination of NH₄-N and NO₃-N (Jackson, 1973).

Ammoniacal and nitrate nitrogen in water sample can be determined titrimetrically after distilling ammonia in boric acid. This method has an advantage that the estimation of both NH₄-N and NO₃-N can be done in the same aliquot.

Reagents

- Sodium hydroxide solution 40% by dissolving 400 g NaOH in distilled water and volume made to 1 litre.
- Mixed indicator solution by dissolving 0.1 g methyl red and 0.5 g bromocresol green in ethyl alcohol and volume made to 100 ml.
- Boric acid – indicator solution by dissolving 20 g boric acid in 900 ml hot distilled water. After cooling 20 ml of mixed indicator is added and volume made to 1 litre with distilled water.
- Standard sulphuric acid solution 0.02 N
- Devarda’s alloy (50 Cu:5 Al:5 Zn)
Procedure

Taken 50 ml water sample into distillation flask and 20 ml boric acid – indicator solution in 150 ml Erlenmeyer flask. This flask was so connected with the condenser that the tip of the condenser was dipped into boric acid solution. Added 10 ml NaOH solution into the distillation flask. Stoppered the flask and contents were steam distilled into boric acid solution till the 40 ml distillate was collected and the color of the distillate became green. Titrated the distillate with 0.02 N H₂SO₄ solution till the appearance of pinkish color. A blank was also run with distilled water. The NH₄-N contents were then calculated in terms of mg L⁻¹.

For determining NO₃-N, nitrates are reduced to ammonia by Devarda’s alloy. After determination of NH₄-N, the stopper of the distillation flask was removed and 0.2 g Devarda’s alloy was added, replaced the stopper and ammonia was redistilled into fresh 20 ml boric acid solution and titrated against standard acid as was done in case of NH₄-N. The NO₃-N contents were calculated as mg L⁻¹.

3.5. 11 Determination of micronutrients and metals.

The elemental analysis of sewage effluent water was done as per the procedure as described by Hundal and Sandhu (1990). A thoroughly mixed sample of water was taken into 1 litre beaker. After adding 5 ml conc. HNO₃, the contents were evaporated to near dryness on a water bath. The cooled contents were digested with HNO₃ and HClO₄ mixture (5:1) on a hot plate. On completion of digestion the residue was diluted with double distilled water and made to the volume, filtered and analyzed for micronutrients and metal
contents (Lindsay and Norwell, 1978) with Atomic Absorption Spectrophotometer model EC-4129 using appropriated hollow cathode lamps.

### 3.6 Physical analysis of soil

#### 3.6.1 Soil particle size distribution analysis by pipette method (Black, 1965)

Soil freed from organic matter and bases by treatment with $\text{H}_2\text{O}_2$ and by leaching with dilute acid is homogenized in the presence of a dispersing agent to achieve complete dispersion of the soil particles. The dispersed soil-water suspension is transferred to a tall cylinder and diluted to 1 litre with distilled water. After thorough mixing of the suspension the cylinder is placed on a firm table to allow sedimentation of the mineral particles. Mineral particles of different diameter settle at different velocities. It is possible to estimate in how much time, the particle of a diameter would settle to a pre-determined depth.

**Equipments and reagents**

- Buchner funnel, Suction flask, suction pump, mechanical homogenizer, tall cylinders 1000 ml steam bath, stop watch and oven.
- Glacial acetic acid, $30\% \text{ H}_2\text{O}_2$, $0.1 \text{ N HCl}$, $0.5\%$ Phenolphthalein indicator, $0.1\text{N NaOH}$ and $5\%$ sodium hexametaphosphate solution.

**Procedure**

The samples were dried by spreading under shade for 3-4 days. Fifty g soil sample was passed through 2 mm sieve. Soil portion passed through 2 mm sieve was retained for particle size distribution analysis. Ten grams of sieved soil samples in duplicate were taken in tall beakers, 20 ml distilled water added and acidified with $0.1\text{N HCl}$. Contents were stirred with glass rod and 5 ml 30
% H₂O₂ was added. Contents stirred until reaction due to evolution of CO₂ subsides. Another 5 ml portion of H₂O₂ added and contents were stirred. Beakers were covered with watch glass and left in an oven at 70 °C for overnight. Next day, added 20 ml H₂O₂ and stirred the contents for 10 minutes, made alkaline with 0.01 N NaOH, placed on hot plate and brought to boil for 30 minutes to expel excess of H₂O₂ added. Beakers were then allowed to cool. This treatment removes organic carbon from the soil.

Whatman No. 42 filter paper was sized to fit properly in buchner funnel and set it properly. Buchner funnel fitted on suction flask and connected to suction pump. Transferred the soil-water suspension into the buchner funnel and soil was leached with three successive 25 ml of 0.1 N HCl. Thereafter, the soil was leached with 125 ml of distilled water in portions of 25 ml. Soil of buchner funnel was then transferred into a preweighed aluminum container, dried at 105 °C for 24 hours and after cooling its weight was recorded.

Transferred other sample of H⁺ saturated soil into a 1 litre tall beaker with the help of glass rod with rubber polcieman. The filter paper was washed and washing was also collected in the beaker. Added to it 0.1 N NaOH to make the suspension alkaline to phenolphthalein. Then 10 ml of 5% sodium hexametaphosphate solution was added into the beaker and stirred. Suspension of the beaker was transferred into dispersion cup of homogenizer, added distilled water to fill the cup up to 3.75 cm below the rim. The suspension was homogenized with motor for 10 minutes for complete dispersion of soil particle. The contents of the dispersion cup were transferred into 1000 ml soil testing cylinder. Washings of the dispersion cup was also transferred to the
same cylinder. The volume of the cylinder was made to 1000 ml with distilled water. The contents of the cylinder was thoroughly mixed for 60 seconds by turning the cylinder to upside down closing the mouth with a rubber stopper. The cylinder was then placed on a firm table. To initiate the sedimentation the stop was started. Twenty seconds before the predetermined time, lowered a 10 ml pipette into the suspension up to 10 cm depth and withdrawn 10 ml aliquot for collecting particles less than 20 microns in diameter. Transferred into preweighed crucible and pipette was also washed into the same crucible. Similarly drawn another 10 ml aliquot from the suspension from same depth for collecting particles less than 2 microns in diameter. Transferred the aliquot in another reweighed crucible. Time of sampling was noted from the table provided for this purpose.

Now both the crucible were placed in an oven at 105°C and evaporated the contents to a constant weight. Weight of the crucibles were recorded after cooling. From these, the sand, silt clay and percentage were calculated.

3.6.2 Measurement of particle density (Dp) of soil (Gupta, 2002).

The mass of unit volume of soil solid is called particle density Dp. It is determined by measuring the mass and volume of soil solids. The pycnometer was weighed and then filled with water completely and again weighed. Ten g soil is taken in a beaker and boiled with small quantity of water to expel trapped air. The pycnometer was emptied and the soil from beaker was transferred to it with a jet of water. After cooling at room temperature the pycnometer was filled with water completely and its weight was recorded. The
weight of the soil divided by the weight of the water displaced gives true density of the soil. The Dp was then calculated from these values.

3.6.3 Measurement of bulk density (Db) of (disturbed) soil (Gupta, 2002).

The bulk density or apparent specific gravity of soil is the mass of a unit volume of soil bulk including pore space.

Weighed large weighing bottle of 50 ml capacity without stopper. This bottle was filled with soil upto brim by tapping the bottle perfectly and bottle weight was recorded. The soil was then removed and bottle was filled with water using a burette and exact volume of water to fill the bottle was noted. The Db was obtained by dividing the weight of soil with volume of soil (volume of water needed to fill the bottle).

3.6.4 Measurement of the porosity of the soil

The porosity of the soil is the fraction of soil volume not occupied by soil particles. It was measured with the help of Db and Dp using following relationship.

\[
\text{per cent pore space} = 100 - \left( \frac{\text{Db}}{\text{Dp}} \right) \times 100
\]

3.7 Chemical analysis of soils.

Assessment of soil’s fertility involves an estimation of its available nutrient status, because only a small fraction of what the soil contains is in the plant - available form and this fraction too is not directly proportional to the total nutrient content of the soil (Tandon, 1993).
Collected soil samples were analyzed using standard, commonly-employed methods for the estimations of pH, EC, CEC, N, P, K, OC, Na, Ca, Mg, micronutrients and metals.

3.7.1 Determination of pH (Jackson, 1973).

Transferred 20 g air dried soil sample into 100 ml beaker and added 40 ml distilled water, stirred the suspension with a glass rod intermittently for 30 minutes. After calibration of pH meter with standard buffers of 4.0 and 9.2 pH, immersed the electrodes into soil water suspension and recorded the pH.

3.7.2 Determination of electrical conductivity (Chapman and Pratt, 1961).

The soil water suspension after measuring the pH, was used to determine the EC. After calibrating the conductivity meter, the conductivity cell was dipped into the suspension, read the conductivity values in dS m⁻¹ directly from the digital meter.

3.7.3 Cation exchange capacity by saturation with NH₄⁺ ions (Amma, 1989).

The exchange sites in the soil was first saturated with NH₄⁺ ions by treatment of the soil with 1 N ammonium acetate (pH 7.0). Residual electrolytes were then removed by leaching with 95% ethanol. The leachate containing NH₄⁺ is collected into a volumetric flask and made to the volume. An appropriate quantity from the collect was then distilled by Kjeldahl distillation method into 2% Boric acid solution containing mixed indicator. The boric acid containing absorbed NH₄⁺ was then titrated with standard acid and Milliequivalent of exchangeable NH₄⁺ of the soil was calculated and reported in terms of me of NH₄⁺ / 100 g soil.
3.7.4 Determination of available nitrogen (Subbiah and Asija, 1956).

The procedure involves distilling the soil with alkaline potassium permanganate solution and determining the ammonia liberated. This serves as an index of available (mineralizable) nitrogen status of a soil.

Reagents

- KMnO₄ Solution 0.32% by dissolving 3.2 g of reagent grade KMnO₄ in distilled water and volume made to 1 litre.
- NaOH solution 2.5% by dissolving 25 g NaOH flakes in distilled water and volume made to 1 litre.
- Liquid paraffin
- Standard H₂SO₄ 0.02 N solution
- Mixed indicator solution by dissolving 0.1 g methyl red and 0.5 g bromocresol green in ethyl alcohol and volume made to 100 ml.
- Boric acid indicator solution by dissolving 20 g boric acid in 700 ml hot distilled water. After cooling 200 ml ethanol and 20 ml mixed indicator solution added to it. Slowly added few ml of 0.05 N NaOH solution until the color becomes reddish purple. Diluted the solution to 1 litre with distilled water.

Procedure

Twenty gram soil was taken into 800 ml Kjeldahl’s flask. After adding 20 ml distilled water the contents were swirled and then 1 ml of liquid paraffin and few glass beads were added to prevent frothing and bumping during distillation. Then added 100 ml each of 0.32% KMnO₄ and 2.5 NaOH solutions. Distilled the contents in a distillation assembly and collected the
liberated ammonia in a 250 ml Erlenmeyer flask containing 20 ml of boric acid solution with mixed indicator. Upon distillation the pink color of boric acid turned green due to absorption of ammonia. Nearly 100 ml distillate was collected in 30 minutes. The contents were then titrated with 0.02 N H₂SO₄ to original pink color. A blank without soil was also run simultaneously. Calculations were done and results were expressed in terms of N kg ha⁻¹.

3.7.5 Determination of available phosphorus (Watanabe and Olsen, 1965).

Reagents

- Sodium bicarbonate (NaHCO₃) 0.5 M (pH 8.5) solution by dissolving 84 g NaHCO₃ in water and made up the volume to 2 litres with distilled water. The pH of the solution was adjusted to 8.5 with 1M NaOH solution.

- Reagent A- by dissolving 12 g ammonium molybdate in 250 ml distilled water. Dissolved 0.2908 g antimony potassium tartarate in 100 ml of distilled water. These two solutions were mixed and volume made up to 2 litres.

- Reagent B- by dissolving 1.056 g ascorbic acid in 200 ml of reagent A.

- Sulphuric acid solution 2.5 M

- Standard stock solution of P by dissolving 0.4393 g AR grade KH₂PO₄ in distilled water and to this 25 ml of 7 N H₂SO₄ was added and volume made up to 1 litre with distilled water. This gave 100 ppm P solution.

- Working solution of 2 ppm P prepared by diluting known portion of 100 ppm P solution to 50 times.
Procedure

To prepare the standard curve 1, 2, 3, 4, 5, and 10 ml of 2 ppm P solution was taken in 25 ml volumetric flask. Added to these 5 ml sodium bicarbonate solution, acidified with 5 ml of 2.5 M H_2SO_4, and added distilled water to make the volume to nearly 20 ml and then 4 ml of reagent B was added. The volume was made up to 25 ml with distilled water. After 10 minutes read the intensity of blue color developed in a Photoelectric colorimeter at 660 nm. The values of absorbance were plotted against different concentrations of P and concentration of P in ppm was calculated from the straight line curve.

Weighed 2.5 g soil into 150 ml Erlenmeyer flask added ¼ teaspoonful of Darco G 60 P free activated charcoal and then 50 ml sodium bicarbonate solution was added. The contents were shaken for 30 minutes on a reciprocating shaker. A blank was also run without soil. After shaking, the contents were filtered through Whatman No. 42 filter paper into a beaker. Five ml aliquot from this extract was taken into 25 ml volumetric flask, acidified with H_2SO_4, added distilled water, 4 ml of reagent B and made to volume. The intensity of the blue color developed was read on the colorimeter and concentration of P in ppm was calculated from the curve and reported in terms of available P kg ha\(^{-1}\).

3.7.6 Determination of available Potassium (Chapman and Pratt, 1961).

The term available K refers to exchangeable plus water soluble K. The K is extracted with neutral normal ammonium acetate solution from soil followed by filtration and determination using a Flame photometer.
Reagents

- Neutral N ammonium acetate solution by dissolving 154 g ammonium acetate (\(\text{CH}_3\text{CONH}_3\)) in water (1.8 litre) and adjusting pH to 7.0 with dilute \(\text{NH}_4\text{OH}\) or \(\text{CH}_3\text{COOH}\). The volume was made to 2 litre with distilled water.

- Potassium Chloride (KCl) standard stock solution of 1000 ppm K by dissolving 1.908 g of AR grade KCl in distilled water and diluting up to 1 litre.

- Working standard solution of 100 ppm K by diluting 100 ml of 1000 ppm K solution to 1 L using neutral normal ammonium acetate.

Procedure

Taken 0, 5, 10, 15 and 20 ml of 100 ppm working solution into 100 ml volumetric flask and volume made using neutral normal ammonium acetate solution. This gave 0, 5, 10, 15 and 20 ppm K solution respectively.

Five gram soil was taken into 150 ml Erlenmeyer flask and added 25 ml neutral normal ammonium acetate solution. The contents were then shaken on a reciprocating shaker for 5 minutes and filtered through Whatman No. 1 filter paper. The instrument was calibrated by spraying with 0 and 20 ppm K solution in reading range of 0-100 on scale. The different concentrations of K in the extract were calculated after calibration of the instrument and by plotting a curve between Flame photometer readings against K concentrations. The K concentration in ppm was calculated from the straight line curve and reported in terms of available K kg ha\(^{-1}\).
3.7.7 Determination of organic carbon (Walkley and Black, 1934).

Organic matter in the soil is oxidized with a mixture of potassium dichromate and concentrated sulphuric acid utilizing the heat of dilution of sulphuric acid. Unutilized potassium dichromate is back-titrated with ferrous ammonium sulphate.

Reagents

- Potassium dichromate (\(K_2Cr_2O_7\)) 1 N by dissolving 49.04 g of pure \(K_2Cr_2O_7\) in distilled water and volume made up to 1 litre.
- Ferrous ammonium sulphate (\(FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O\)) 0.5 N by dissolving 140 g of FAS in about 800 ml distilled water and 20 ml of concentrated \(H_2SO_4\). Cooled the solution and volume made to 1 litre with distilled water.
- Diphenylamine indicator solution by dissolving 0.5 g of diphenylamine in mixture of 20 ml water and 80 ml of concentrated \(H_2SO_4\).
- Orthophosphoric acid 85%.

Procedure

Taken 1 g air dried soil into a 500 ml Erlenmeyer flask. One blank is also prepared without soil sample. Added 10 ml of Potassium dichromate solution, swirled the flask and placed them on glazed tiles. Added 20 ml concentrated \(H_2SO_4\) directly into the flask. After swirling the flask again 2-3 times, allowed the flask to stand on tiles for 30 minutes. After 30 minutes added 200 ml distilled water, 10 ml of orthophosphoric acid and 1 ml of diphenylamine indicator. Titrated the contents with ferrous ammonium sulphate.
solution till the color changes from blue-violet to green. Calculated the organic carbon in per cent from values obtained in titration.

3.7.8 Determination of Na, Ca and Mg (Gupta, 2002).

A 1:2 soil water extract is prepared by weighing 100 g soil in a 500 ml Erlenmeyer flask and added to it 200 ml of distilled water. The contents were shaken for 30 minutes on a reciprocating shaker, filtered the suspension through Whatman No. 1 filter paper and collected the clear filtrate which was used for determination of Na, Ca and Mg.

3.7.8.1 Determination of Na

Ten milliliter of aliquot of soil-water extract was taken in a 100 ml volumetric flask and made to the volume with distilled water. The Na contents were determined after calibration of Flame photometer in accordance with the procedure explained earlier for the determination of Na in water samples. The contents were calculated in terms of mg L$^{-1}$ from the calibration curve.

3.7.8.2 Determination of Ca and Mg

Ten milliliter of soil-water extract is taken in a 250 ml Erlenmeyer flask and Ca contents were determined by the procedure explained earlier for the determination of Ca in water samples using Murexide indicator then Ca + Mg using EBT indicator. To obtain the values of Mg, the value of Ca is subtracted from the values of Ca + Mg. The results so obtained were recorded in terms of mg L$^{-1}$.
3.8 Determination of micronutrients and metals (Lindsay and Norwell, 1978).

The method consists of use of DTPA (Diethylene triamine penta acetic acid) as an extractant which has been widely accepted for simultaneous extraction of micronutrients and metals. The DTPA extractable micronutrients and metals are considered as available to plants. The DTPA is a chelating agent that combines with free metal ions in solution to form soluble complexes. Triethanolamine (TEA) is used as a buffer because it burns cleanly during atomization. The DTPA has capacity to complex with metal ions as 10 times of its molecular weight. The capacity ranges from 550 to 650 mg kg\(^{-1}\) (Gupta, 2002). The contents of micronutrients and metals were determined on an Atomic Absorption Spectrophotometer model EC 4129 using appropriate hollow cathode lamps.

**Reagents**

Extracting solution 0.005 M DTPA, 0.01 M CaCl\(_2\).2H\(_2\)O and 0.1 M TEA adjusted to pH 7.3 by dissolving 1.967 g DTPA and 13.3 ml TEA in about 400 ml double distilled water. To this added 1.47 g CaCl\(_2\).2H\(_2\)O dissolved in about 500 ml double distilled water. Now added DTPA – TEA mixture to it, mixed thoroughly and adjusted the pH to 7.3 using 1 N HCl, made to the volume of 1 litre.

Standard solution (1) 1000 ppm (E-Merck, Germany) solution of corresponding element.
Standard solution (2) Ten ml of 1000 ppm solution was taken into 100 ml volumetric flask and volume made up to the mark with DTPA extracting solution to get 10 ppm solution of the corresponding element.

Working standard solution – 0, 1, 2, 4, 6 and 8 ml of 10 ppm standard solution was taken to a series of 100 ml volumetric flasks and diluted each to the mark with DTPA extracting solution. This gave a concentration of 0, 0.1, 0.2, 0.4, 0.6 and 0.8 ppm of working solution.

Procedure

Weighed 20 g air dried soil sample into 150 ml Erlenmeyer flask and added 40 ml DTPA extracting solution. The flasks were stoppered and shaken on a reciprocating shaker for 2 hours. After shaking, the contents were filtered into polypropylene bottles using Whatman No. 1 filter paper. A blank without soil was also run in the similar way. The filtrate was retained for analysis of micronutrients and metals with AAS model EC 4129.

The instrument was set to zero with blank and fed the serial working standards selecting the hollow cathode lamp for the element to be determined after setting of lamp current and corresponding wavelength. The AAS was standardized to read the absorbance and concentration of the given standards and a linear relationship was observed. Then the DTPA-extracts of the samples were fed and the absorbance and concentrations of the elements present was recorded. The AAS displays the concentration of the element in ppm directly, so the concentration of the element in the soil extract was calculated by multiplying the displayed reading by dilution factor which was 2 in all these estimations.
Three standards of each element were also run intermittently to check the reproducibility of the results.

The contents of micronutrients and metals were calculated and expressed in terms of ppm or mg kg⁻¹.

3.9 Chemical Analysis of plants

Widely accepted methods for the determination of macro and micronutrients have been adapted for analyzing plant materials. The plant materials have been analyzed for total contents of N, P and K along with micronutrients and heavy metal contents.

3.9.1 Determination of Nitrogen.

The total nitrogen in the plant samples was determined by Kjeldahl’s method. An automatic N digestion and distillation unit KEL-PLUS, KES-20 L was used in the determination.

Procedure

Taken 0.5 g powdered plant sample and placed into digestion tubes, added 10 ml conc. H₂SO₄ and 1 g catalyst mixture containing K₂SO₄ and CuSO₄ in 10:1 ratio. Heating started and the digestion was complete in nearly 2-3 hours when temperature of the digestion block reached to 400 °C. After completion of the digestion the tubes were removed and allowed to cool. After cooling 50 ml water was added and digestion tubes were connected to the distillation unit. The alkali pump was set to deliver 30 ml of 40 % NaOH. The distillation unit was started and nearly 50 ml distillate was collected in a conical flask containing 20 ml of 2% boric acid with mixed indicator. Liberated
ammonia was absorbed in boric acid which was back titrated with 0.05 N 
H₂SO₄. Percentage of N in the plant samples was then calculated.

3.9.2 Determination of Phosphorus.

Vanadate, molybdate and orthophosphates react together to give a 
yellow color complex in HNO₃ medium. The main advantage of this method is 
stability of color and freedom from the interference by wide range of ionic 
species in concentrations upto 1000 ppm (Tandon, 1993).

Reagents

Ammonium molybdate – ammonium vanadate solution in HNO₃ by 
dissolving 22.5 g (NH₄)₆Mo₇O₂₄·4H₂O in 400 ml distilled water and 1.25 g 
ammonium vanadate in 300 ml boiling water. The vanadate solution was then 
added to molybdate solution and cooled at room temperature. After cooling 250 
ml of conc. HNO₃ was added and diluted to 1 litre.

Standard solution of phosphorus 50 ppm by dissolving 0.2195 g AR grade 
KH₂PO₄ and diluting to 1 litre with distilled water.

Procedure

Taken 0.5 g powdered plant sample and placed into digestion tubes, 
added 10 ml of acid (Perchloric acid and Nitric acid) mixture in 9:4 ratios was 
added. The plant material was digested till the liquid became clear. This 
process was completed within 2-3 hours. The digestion tubes were removed 
cooled to room temperature and 20 ml of distill water was added. Contents 
were filtered through Whatman No. 1 filter paper by 3-4 washings of the tube 
with distilled water and finally volume was made up to 100 ml using distilled 
water.
Transferred 0, 1, 2, 3, 4 and 5 ml of standard solution of 50 ppm P into 50 ml volumetric flask to get 0, 1, 2, 3, 4 and 5 ppm of P solution. Added 10 ml vanadomolybdate reagent and made to 50 ml volume using distill water. Read the absorbance of solution after 30 minutes at 420 nm with a Colorimeter using blue filter and drawn the calibration curve. The concentration of P in plant sample was calculated from the curve, by development of yellow color taking suitable aliquot of plant digest and contents were expressed as per cent P in plants.

3.9.3 Determination of Potassium

The samples digested by diacid mixture (as under phosphorus) were used for determination of potassium after preparation of calibration curve, as described under determination of potassium (under soil chemical analysis using) a Flame photometer.

3.9.4 Determination of micronutrients and metals.

The samples digested by diacid mixture as described under phosphorus were used for determination of micronutrients and metals after preparation of calibration curve as detailed previously under determination of micronutrients and metals in soils using Atomic Absorption Spectrophotometer model EC 4129.
REFERENCES


